

学校编码：10384

分类号_____密级_____

学号：B20051403130

UDC _____

厦门大学

博 士 学 位 论 文

中国深圳海域及九龙江口细菌生态特征研究

**Studies on the Characteristics of Bacterial Ecology in
Shenzhen Coastal Waters and Jiulong River Estuary, China**

陈明霞

指导教师姓名：郑天凌 教授

李和阳 副研究员

专 业 名 称：微 生 物 学

论文提交日期：2010 年 8 月

论文答辩时间：2010 年 12 月

学位授予日期：

答辩委员会主席：_____

评 阅 人：_____

2010 年 9 月

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果,均在文中以适当方式明确标明,并符合法律规范和《厦门大学研究生学术活动规范(试行)》。

另外,该学位论文为()课题(组)的研究成果,获得()课题(组)经费或实验室的资助,在()实验室完成。(请在以上括号内填写课题或课题组负责人或实验室名称,未有此项声明内容的,可以不作特别声明。)

声明人(签名):

年 月 日

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

（ ） 1. 经厦门大学保密委员会审查核定的保密学位论文，
于 年 月 日解密，解密后适用上述授权。

（ ） 2. 不保密，适用上述授权。

（请在以上相应括号内打“√”或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年 月 日

摘要.....	1
ABSTRACT	3
第一章 绪论	6
第一节 海洋微生物的生态研究	6
1.1.1 海洋微生物的研究简史.....	6
1.1.2 海洋微生物在海洋生态系统中的作用	8
1.1.3 环境微生物生态学研究方法及研究进展.....	11
第二节 海洋弧菌的生态研究	17
1.2.1 弧菌的分类地位及特征.....	17
1.2.2 弧菌生态分布及生态意义	18
1.2.3 海洋弧菌生态分布的影响因素	26
1.2.4 弧菌的“活的不可培养状态”	28
1.2.5 弧菌与其它生物之间的关系	29
第三节 细菌在海洋环境监测及评价中的应用	32
1.3.1 细菌总数	33
1.3.2 大肠菌群和粪大肠菌群.....	33
1.3.3 弧菌数量	34
第四节 细菌的耐药性概况	34
1.4.1 细菌对 β -内酰胺类抗生素的耐药性	35
1.4.2 细菌对四环素类的耐药性	35
1.4.3 细菌对氟喹诺酮类的耐药性	35
第五节 本论文的研究思路	36
1.5.1 深圳海域弧菌种类组成、数量分布及其与环境相互关系研究.....	36
1.5.2 九龙江口 TCBS 类群的分布及其耐药性研究	38

第二章 材料与方法	41
2.1 材料	41
2.2 基本方法	48
2.3 深圳海域水体及九龙江口沉积物TCBS类群(可培养弧菌)多样性分析	56
2.4 深圳海域水体未培养弧菌多样性分析	58
2.5 细菌药敏实验	59
2.6 数据统计分析	60
第三章 结果与分析	61
第一节 深圳海域弧菌种类、数量分布及其与环境相关关系研究	61
3.1.1 环境理化因子及生物因子分析	61
3.1.1.1 主要水质参数	61
3.1.1.2 叶绿素 a (Chlorophyll-a, Chl-a) 分布	66
3.1.1.3 细菌总数及可培养细菌总数分布	68
3.1.1.4 粪大肠菌群分布	72
3.1.2 可培养弧菌种类、数量分布及其与环境相关关系研究	74
3.1.2.1 可培养弧菌的数量分布及其与环境的关系	74
3.1.2.1.1 2008 年 4 月航次可培养弧菌的数量分布及其与环境的关系	74
3.1.2.1.2 2008 年 9 月航次可培养弧菌的数量分布及其与环境的关系	77
3.1.2.1.3 小结	80
3.1.2.2 可培养弧菌种类的分布及其与环境的关系	80
3.1.2.2.1 可培养弧菌及相关类群 (TCBS 类群) 鉴定方法的构建	80
3.1.2.2.2 2008 年 4 月深圳海域可培养弧菌种类分布及其与环境的关系	82
3.1.2.2.2.1 各站位可培养弧菌种类组成及分布	82
3.1.2.2.2.2 可培养优势弧菌类群的分布及其与环境之间的关系	92
3.1.2.2.2.3 小结	97
3.1.2.2.3 2008 年 9 月深圳海域可培养弧菌种类分布及其与环境的关系	98
3.1.2.2.3.1 各站位可培养弧菌种类组成及分布	98
3.1.2.2.3.2 可培养优势弧菌类群的分布及其与环境之间的关系	105

3.1.2.2.3.3 小结	107
3.1.3 未培养弧菌种类分布及其与环境相关关系研究	110
3.1.3.1 水体样品总 DNA 的提取.....	110
3.1.3.2 样品中细菌 16S rDNA 序列扩增.....	110
3.1.3.3 弧菌特异性 16S rDNA 序列扩增.....	111
3.1.3.4 弧菌特异 16S rDNA 的 RFLP 分析.....	111
3.1.3.5 未培养弧菌的种类分布及其与环境的关系	117
3.1.3.6 小结.....	121
第二节 九龙江口沉积物 TCBS 类群的分布及其耐药性研究	122
3.2.1 九龙江口沉积物 TCBS 类群的组成及分布	122
3.2.1.1 九龙江口沉积物TCBS类群的组成	122
3.2.1.2 各站位 TCBS 类群的分布.....	125
3.2.1.3 不同 TCBS 类群的分布特征.....	126
3.2.2 TCBS 菌株的耐药性研究.....	129
3.2.2.1 TCBS 菌株的药敏结果.....	129
3.2.2.2 不同 TCBS 类群的耐药状况及分布	133
3.2.2.3 不同抗生素的耐药分析.....	136
3.2.3 小结.....	137
第四章 讨论	138
第一节 深圳海域弧菌的分布特点及其与环境的关系.....	138
4.1.1 可培养弧菌总量时空变化及其与环境的关系	138
4.1.2 深圳海域可培养弧菌种类组成及季节变化.....	143
4.1.3 深圳海域可培养弧菌与未培养弧菌组成及分布比较.....	146
第二节 关于环境中弧菌来源的讨论.....	147
4.2.1 TCBS培养基 (Thiosulfate Citrate Bile Salts Sucrose Agar, 硫代硫酸盐-柠檬酸盐-胆盐-蔗糖琼脂)、TCBS菌群和弧菌的关系.....	147
4.2.2 海洋环境中弧菌的来源.....	148
第三节 细菌学研究与环境评价的几点讨论	152

4. 3. 1 弧菌在环境细菌学监测及评估中的作用.....	152
4. 3. 2 水体弧菌数量及种类组成与养殖动物病害的关系.....	153
4. 3. 3 深圳海域细菌总数及可培养细菌总数反映的环境问题.....	154
第四节 关于细菌耐药性的讨论	158
4. 4. 1 九龙江口细菌的耐药性.....	158
4. 4. 2 细菌耐药性的控制策略.....	160
总结与展望.....	161
参考文献	163
附录一 已完成的学术论文及参与的科研课题	193
附录二 pMD18-T vector 图谱.....	194
附录三 DNA 分子量标准	195
附录四 <i>Vibrio</i>, <i>Photobacterium</i>, <i>Aeromonas</i>, <i>Plesiomonas</i>, 及相关种类在 <i>Bergey's Manual</i> 不同版本 (1957 - 2001) 中的归类差异.....	196
附录五: 本文中细菌的拉丁名及中文名称.....	197
致谢	199

CONTENTS

Chinese abstract	1
English abstract	3
Chapter 1 Introduction	6
Part 1 Ecology of marine microbiology	6
1.1.1 History of marine microbiology	6
1.1.2 Function of microbiology in marine ecosystem	8
1.1.3 Development of the methods and techniques of environmental microbiology	11
Part 2 Ecology of marine vibrios	17
1.2.1 Classification and characteristics of vibrios.....	17
1.2.2 Occurrence and importance	18
1.2.3 Enviromental factors influence the distribution of marine vibrios	26
1.2.4 Viable but nonculturable state of vibrios.....	28
1.2.5 Relationship between vibrios and other organisms	29
Part 3 Application of bacteria in marine enviromental monitoring and assessment ...	32
1.3.1 Total bacteria	33
1.3.2 Coliforms and fecal coliforms	33
1.3.3 Quantity of vibrios.....	34
Part 4 Present of bacterial antibiotic resistance	34
1.4.1 Resistance to β -lactam antibiotics.....	35
1.4.2 Resistance to tetracycline antibiotics	35
1.4.3 Resistance to fluoroquinolone antibiotics.....	35
Part 5 Design, purpose and significance of the thesis	36
1.5.1 Composition and distribution of vibrios and their correlation with environmental factors in Shenzhen coastal watersv	36
1.5.2 Antibiotic resistance and distribution of TCBS groups from sediments of Jiulong River	

estuary.....	38
Chapter 2 Materials and methods	41
2.1 Materials.....	41
2.2 Basic methods	48
2.3 Diversity of TCBS groups (culturable vibrios) in Shenzhen coastal waters and sediments of Jiulong River estuary.....	56
2.4 Diversity of uncultured vibrios in Shenzhen coastal waters	58
2.5 Bacterial antibiotic sensitivity test	59
2.6 Statistics analysis	60
Chapter 3 Results and analysis	61
Part I Composition and distribution of vibrios and their correlation with environmental factors in Shenzhen coastal waters	61
3.1.1 Analysis of environmental factors.....	61
3.1.1.1 Physic-chemical factors.....	61
3.1.1.2 Distribution of chlorophyll- <i>a</i> (chl- <i>a</i>)	66
3.1.1.3 Distribution of total and culturable bacteria	68
3.1.1.4 Distribution of fecal coliforms	72
3.1.2 Composition and distribution of culturable vibrios and their correlation with environmental factors	74
3.1.2.1 Distribution of the abundance of culturable vibrios and the correlation with environmental factors.....	74
3.1.2.1.1 Samples from April 2008	74
3.1.2.1.2 Samples from September 2008	77
3.1.2.1.3 Summary	80
3.1.2.2 Composition and distribution of culturable vibrios and the correlation with environmental factors.....	80
3.1.2.2.1 Methods for identification of culturable vibrios and related species (TCBS groups).	80
3.1.2.2.2 Samples from April 2008	82
3.1.2.2.2.1 Composition of the culturable vibrios in each station	82

3.1.2.2.2 Correlationship between dominant culturable <i>Vibrio</i> sp. and environmental factors	92
3.1.2.2.3 Summary	97
3.1.2.2.3 Samples from September 2008.....	98
3.1.2.2.3.1 Composition of the culturable vibrios in each station	98
3.1.2.2.3.2 Correlationship between dominant culturable <i>Vibrio</i> sp. and environmental factors.....	105
3.1.2.2.3.3 Summary	107
3.1.3 Composition and distribution of the uncultured vibrios and their correlation with environmental factors	110
3.1.3.1 Total DNA extraction from water samples	110
3.1.3.2 16S rDNA PCR amplification of total DNA.....	110
3.1.3.3 PCR amplification of <i>Vibrio</i> -specific 16S rDNA	111
3.1.3.4 <i>Vibrio</i> -specific 16S rDNA -RFLP analysis.....	111
3.1.3.5 Composition and distribution of the uncultured vibrios and their correlation with environmental factors	117
3.1.3.6 Summary	121
Par 2 Antibiotic resistance and distribution of TCBS groups from sediments of Jiulong River estuary	122
3.2.1 Composition and distribution of TCBS groups in sediments of Jiulong River estuary.	122
3.2.1.1 Composition of TCBS groups in sediments of Jiulong river estuary.....	122
3.2.1.2 Distribution of TCBS groups in each station.....	125
3.2.1.3 Distribution pattern of each TCBS group.....	126
3.2.2 Antibiotic resistance of TCBS strains.....	129
3.2.2.1 Results of antibiotic sensitivity test	129
3.2.2.2 Antibiotic resistance pattern of each TCBS group.....	133
3.2.2.3 Resistance pattern of all TCBS groups to each antibiotic	136
3.2.3 Summary	137

Chapter 4 Discussion	138
Part 1 Composition and distribution of vibrios and their correlation with environmental factors in Shenzhen coastal waters	138
4.1.1 Seasonal distribution of the abundance of the culturable vibrios and the correlation with environmental factors	138
4.1.2 Composition and seasonal distribution of the culturable vibrios	143
4.1.3 Comparison between culturable and uncultured vibrios	146
Part 2 Discussion of the source of marine vibrios.....	147
4.2.1 TCBS agar (Thiosulfate Citrate Bile Salts Sucrose Agar), TCBS groups and vibrios...	147
4.2.2 The source of marine vibrios	148
Part 3 ABC about bacteriology and environmental assessment.....	152
4.3.1 Vibrios in environmental monitoring and assessment.....	152
4.3.2 Relationship between the vibriosis of aquaculture animals with the composition and abundance of vibrios	153
4.3.3 Relationship between the total and culturable bacteria in Shenzhen coastal waters and environmental assessment	154
Part 4 Discussion of bacterial antibiotic resistance	158
4.4.1 Antibiotic resistance of TCBS strains from Jiulong River estuary	158
4.4.2 Methods to control bacterial antibiotic resistance.....	160
Conclusion and prospect.....	161
References	163
Appendix	
1. Papers accepted, submitted and in preparing and Projects participated in.....	193
2. pMD18-T vector profile.....	194
3. DNA Markers	195
4. Different classifications of <i>Vibrio</i> , <i>Photobacterium</i> , <i>Aeromonas</i> , <i>Plesiomonas</i> , and related organisms in five editions of <i>Bergey's Manual</i> (1957–2001)	196
5. Latinized scientific name and Chinese name of the bacteria in the paper	197
Acknowledgement.....	199

摘 要

海洋弧菌是海洋微食物环的重要组成部分,对海洋环境营养物的循环起着非常重要的作用。此外,弧菌还是人类及水生动物(包括养殖动物)的重要致病菌。对弧菌及其相关类群的种类组成、数量分布、与环境相关关系以及耐药性的研究,是环境细菌学研究的重要组成部分,其研究对环境健康、疾病防治及生态安全等方面具有重要意义。本文运用传统的分离培养方法和现代的分子分类检测方法,对深圳海域及九龙江口弧菌(包括可培养、未培养的)的生态分布及其与环境相关关系进行研究。

研究内容包括两大部分:

1. 深圳海域弧菌种类组成、数量分布及其与环境相互关系研究
2. 九龙江口 TCBS 类群(生长于 TCBS 琼脂培养基上的细菌类群)的分布及其耐药性研究

主要研究结果包括:

1. 深圳海域可培养弧菌数量分布具有季节性。东部海域弧菌数量春季(2008年4月)(范围为 5.10×10^2 - 4.40×10^4 CFU/mL, 平均为 1.50×10^4 CFU/mL) 高于秋季(2008年9月)(范围为 1.41×10^2 - 2.57×10^3 CFU/mL, 平均为 8.89×10^2 CFU/mL); 西部海域秋季(范围为 0 - 1.56×10^3 CFU/mL, 平均为 5.09×10^2 CFU/mL) 略高于春季(范围为 0 - 6.62×10^2 CFU/mL, 平均为 2.66×10^2 CFU/mL); 弧菌数量高发区出现在4月份大亚湾(4.40×10^4 CFU/mL)。弧菌分布与温度、有机物浓度及盐度等相关,各环境因素互相交联,其相互关系复杂。

2. 珠江口盐度低于11的站位未检测到可培养的弧菌,盐度高于11的站位,可培养弧菌数量随盐度增加而增大。九龙江口盐度较低的上游区域沉积物中未检测到可培养弧菌,而在盐度较高的河口下游区域有弧菌分布,而且其分布比例随盐度增加而增加。

3. 深圳海域清洁水域(YMK001 站位和 GDN064 站位)发现有大量的弧菌存在,且数量高于污染严重的海域(包括深圳湾站位及珠江口下游站位),是弧菌数量的高发区。

4. 深圳海域春季可培养弧菌有 *V. gigantus*、*V. splendidus*、*V. alginolyticus*、*V. cyclitrophicus*、*V. rotiferianus*、*V. mytili*、*V. natriegens*、*V. parahaemolyticus* 和 *V.*

harveyi 的类似种, 其中主要优势类群是 *V. gigantis* 类似种、*V. alginolyticus* 的类似种和 *V. splendidus* 的类似种; 秋季分布有 *V. alginolyticus*、*V. natrieigen*s、*V. mytili*、*V. splendidus* 和 *V. fortis* 的类似种, 其中主要优势类群是 *V. alginolyticus* 类似种和 *V. natrieigen*s 类似种。各类优势弧菌分布都具有季节性: *V. alginolyticus* 类似种是两个季节的优势类群, 其平均数量春季高于秋季; 春季特有的优势类群有 *V. gigantis* 类似种和 *V. splendidus* 类似种, 秋季特有的优势类群有 *V. natrieigen*s 类似种。

5. 未培养弧菌种类包括: *V. pomeroyi*、*V. sinaloensis*、*V. splendidus*、*V. pelagius*、*V. cholerae* 五种弧菌的类似种, 其中 *V. pomeroyi* 类似种是两个月份的优势弧菌类群; *V. sinaloensis* 类似种主要发生于9月份水体温度较高的季节, *V. splendidus* 类似种主要发生于4月份水体温度较低的季节。

6. 九龙江口沉积物(2009年12月)的可培养弧菌类群以 *V. parahaemolyticus* 为主。此外还大量分布有其他TCBS类群, 如 *Shewanella*、*Pseudoalteromonas*、*Pseudomonas*、*Aeromonas*、*Bacillus* 属细菌。深圳海域也同样具有大量非弧菌的TCBS类群存在。弧菌占TCBS菌株总数的比例因不同站位、不同季节而有很大的变化, 其比例为0-100%。盐度较高的区域, 弧菌数量与TCBS菌群数呈直接正相关, 盐度较低的区域弧菌数量与TCBS菌群数无关或关系不明显。

7. 九龙江口TCBS菌株53%对氨苄青霉素耐药, 59%对头孢唑啉耐药, 72%对头孢噻吩耐药, 1%对庆大霉素耐药, 4%对氟哌酸耐药, 3%对四环素耐药, 7%对链霉素耐药。有些菌株为3联、4联或6联耐药菌株。

关键词: 深圳海域, 九龙江口, 弧菌种类组成及数量分布, TCBS类群, 耐药性

Abstract

Vibrios are dominant in the marine environment and play a key role in nutrient cycling in aquatic environments by taking up dissolved organic matter and producing kinds of enzymes. Otherwhile, vibrios are important bacterial pathogens for humans and marine animals, because of which, more and more researchs focus on the composition of vibrios and the relationship with the environmental factors.

Research contents:

1. Composition and distribution of vibrios and their correlation with the environmental factors in Shenzhen coastal waters.
2. Antibiotic resistance and distribution of TCBS groups from sediments of Jiulong River estuary.

Results:

1. Seasonal variations of culturable vibrios in Shenzhen coastal waters were obvious. Within the east coastal waters, the abundance of marine vibrios in Spring (April 2008) (range from 5.10×10^2 CFU/mL to 4.40×10^4 CFU/mL, with an mean value of 1.50×10^4 CFU/mL) was higher than that in Autumn (September 2008) (range from 1.41×10^2 CFU/mL to 2.57×10^3 CFU/mL, with an mean value of 8.89×10^2 CFU/mL), while within the west coastal waters, the abundance of marine vibrios in Autumn (range from 0 CFU/mL to 1.56×10^3 CFU/mL, with an mean value of 5.09×10^2 CFU/mL) was higher than that in Spring (range from 0 CFU/mL to 6.62×10^2 CFU/mL, with an mean value of 2.66×10^2 CFU/mL). The highest number was detected in Daya Bay in Spring (4.40×10^4 CFU/mL). The distribution of vibrios were mainly controlled by temperature, salinity, pH and organic concentration, et al. The relationships between vibrios and environmental factors were complicated and varied depending on different species, locations and seasons.

2. There were no culturable vibrios detected at some Zhujiang river estuary stations where salinity was under 11. Also, There were no culturable vibrios detected in the fresh or brackish stations without the more saline stations of Jiulong river estuary. The results showed that salinity played an important role in the distribution of vibrios

which indicated that vibrios are autochthonous of the marine environment.

3. Vibrios abundance in healthy waters (stations YMK001 and GDN064) was higher than that in badly polluted waters (stations GDN053, GDN057 and GDN063), which showed that vibrios were the natural and important group in the marine ecosystem.

4. In Spring, culturable vibrios in the Shenzhen coastal waters were composed of *V. gigantis*-like species, *V. splendidus*-like species, *V. alginolyticus*-like species, *V. cyclitrophicus*-like species, *V. rotiferianus*-like species, *V. mytili*-like species, *V. natriegens*-like species, *V. parahaemolyticus* -like species and *V. harveyi*-like species, among which *V. gigantis*-like species, *V. alginolyticus*-like species and *V. splendidus*-like species were the three dominant groups. In Autumn, vibrios in the same waters were composed of *V. alginolyticus*-like species, *V. natriegens*-like species, *V. mytili*-like species, *V. splendidus*-like species and *V. fortis*-like species, among which *V. alginolyticus*-like species and *V. natriegens*-like species were the dominant groups. Seasonal variations of each dominant *Vibrio* sp. in the coastal waters was obvious: *V. alginolyticus*-like species was the dominant group in both seasons, the average number of which in April was higher than that in September; *V. gigantis*-like species and *V. splendidus*-like species were dominant in April with lower temperature, while *V. natriegens*-like species was dominant in September with higher temperature.

5. Uncultured vibrios in the Shenzhen coastal waters were composed of *V. pomeroyi*-like species, *V. sinaloensis*-like species, *V. splendidus*-like species, *V. pelagius*-like species and *V. cholerae*-like species, among which *V. pomeroyi*-like species was the main dominant group in both seasons, *V. sinaloensis*-like species was dominant in Autumn with higher temperature, and *V. splendidus*-like species was dominant in Spring with lower temperature. The results indicated that there still were a large number of vibrios in VBNC state.

6. *V. parahaemolyticus*-like species was the only culturable vibrios detected in the sediment of Jiulong River estuary in December 2009. Besides of this, the TCBS groups were composed of *Shewanella* sp., *Pseudoalteromonas* sp., *Pseudomonas* sp., *Aeromonas* sp. and *Bacillus* sp.. And there were non-vibrios species detected in

Shenzhen coastal waters with TCBS agar too. The ratio of the number of vibrios to TCBS strains changed from 0% to 100% depending on different locations and seasons. The number of vibrios showed a positive correlation with that of TCBS strains in more saline sea waters, and no correlation in fresh or brackish waters.

7. The percentage of resistant strains to ampicillin, cefazolin, cephalothin, norfloxacin, gentamicin, tetracycline and streptomycin was 53%, 59%, 72%, 4%, 1%, 3% and 7% respectively. Some strains resisted to 3, 4 or 6 antibiotics.

Key words: Shenzhen coastal waters, Jiulong River estuary, composition and distribution of vibrios, TCBS groups, antibiotic resistance

Degree papers are in the "[Xiamen University Electronic Theses and Dissertations Database](#)". Full texts are available in the following ways:

1. If your library is a CALIS member libraries, please log on <http://etd.calis.edu.cn/> and submit requests online, or consult the interlibrary loan department in your library.
2. For users of non-CALIS member libraries, please mail to etd@xmu.edu.cn for delivery details.

厦门大学博硕士论文摘要库